Center for Macromolecular Crystallography
University of Alabama in Birmingham

Dr. Manuel Navia
Senior Scientist
Vertex Pharmaceuticals,, Inc.

Refinement of Porcine Pancreatic Elastase Using Data from Crystals Grown in Microgravity

Porcine pancreatic elastase (PPE) crystals grown under microgravity conditions on mission STS-26 of the space shuttle "Discovery" have been shown to diffract to considerably higher resolution than the best PPE crystals grown by us on the ground (DeLucas *et. al.* (1989) ***Science*** **246:** 651-654). We have now independently refined both the microgravity and ground-based data. Preliminary results of these refinements are summarized below:

D-MIN RES. to...	EARTH GRAVITY # of RFLS	SHELL R-FACTOR	MICRO-GRAVITY. # of RFLS	SHELL R-FACTOR
3.00Å	3546	19.0%	3624	14.2%
2.50Å	3044	18.8%	322	15.0%
2.00Å	5937	18.7%	6971	14.8%
1.90Å	1751	20.0%	--	--
1.80Å	1873	22.1%	4920	15.7%
1.70Å	1990	26.4%	--	--
1.65Å	944	32.0%	--	--
1.60Å	--	--	6636	17.2%
1.40Å	--	--	8387	20.2%
1.30Å	--	--	2079	26.6%
	EARTH GRAVITY # of RFLS	SHELL R-FACTOR	MICRO-GRAVITY. # of RFLS	SHELL R-FACTOR
TOTAL	19085	19.9%	35841	15.9%

Note: Deviations from ideal bond lengths for both refinements was appx. 0.020Å.

These results show nearly a doubling of experimental diffraction data for this structure, exceeding 1.3Å resolution. Improved phase information derived from the refined structure of PPE based on this microgravity data has allowed us to interpret previously-uninterpretable electron density obtained from ground-based crystals of a complex of PPE with a chemically-reactive inhibitor. Intermediate stages in the enzyme-inhibitor reaction mechanism in the crystal can now be directly observed. Further refinement of PPE structures is in progress.

Table 2:

PROTEIN	a (Å)	b (Å)	c (Å)	angles (deg.)	Soln.
Anhydro-PPE	50.74	57.94	75.28	90. 90. 90.	SUL
A-PPE + peptide	50.94	57.91	75.33	90. 90. 90.	SUL
PPE (gravity)	51.00	58.08	75.29	90. 90. 90.	SUL
PPE (micro-grav)	50.88	58.02	75.35	90. 90. 90.	SUL

Table 3:

PROTEIN	# reflections	resolution	R-Factor	Bond length deviation (Å)	detector type
Anhydro-PPE	17564	1.65Å	15.7%	0.019Å	diffractometer
A-PPE + peptide	16035	1.80Å	16.8%	0.016Å	diffractometer
PPE (gravity)	19085	1.65Å	19.9%	0.016Å	area detector
PPE (micro-grav)	35841	1.30Å	15.9%	0.016Å	area detector